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Original Article

THE DIVERSITY AND ABUNDANCE OF LITTORAL DINOPHYTA AND DINOCYST IN *CHICOCO*-MUD ALONG THE LOWER REACHES OF THE NEW - CALABAR RIVER, NIGER-DELTA, NIGERIA

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ABSTRACT

A total of 15 Chicoco-mud samples were randomly sampled at extreme low neap or spring tides from five Stations located within three communities in Asari-Toru Local Government Area of Rivers State for dinophyta and dinocyst studies, covering wet and dry seasons. The planktological taxonomic system was adopted. Seasonal variations were minimal with low diversity and abundance values, nevertheless, in the wet season, average Margalef's Species Richness Index (d) varied from 0.271 – 0.511, Shannon-Weiner Diversity Index (H) varied from 0.244 – 0.461 bits/Individual (bits/Ind.) and Equitability (E) from 0.193 – 0.988 while in dry season 'd' varied from 0.271 – 0.542, 'H' from 0.092 – 0.471 bits/Ind. and 'E' from 0.193 – 0.988, however, Hutcheson t -test for 'H' between Stations indicated 'significant' and 'not-significant' differences at P < 0.05 level. The most abundant species amongst the dinophyta were the 'resting cyst' producing species of Polykrikos schwartzii Bütschli (Gymnodiniales) and Preperidinium meunieri (Pavillard) Elbrächter (Peridiniales) while for the dinocyst it was Protoperidinium spp. (Peridiniales). Chicoco-mud 'stations' and 'monthly' physicochemical data indicated that Sand, Silt, Clay, Fiber, Wet conductivity, TOC, pH, Avail. P., and NO^-_3 – N were significantly different and affected the diversity and abundance of littoral dinophyta and dinocyst.

***KEYWORDS:** Chicoco-Mud, Diversity, Littoral, Dinophyta, Dinocyst, New-Calabar River & Niger-Delta*

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I. INTRODUCTION

Dinophyta are flagellate eukaryotes (protist) and some species produce resting stages called dinocyst or dinoflagellate cyst as part of their life cycle, however, only 10 to 16% of living dinoflagellates are known to produce cyst (Head, 1996; Matsuako & Fukuyo, 2002). They can occur in all aquatic environments, including in snow and ice and are common in benthic environments (Specter, 1984). Some dinoflagellates are harmful to fisheries due to their ability to bloom in concentrations of more than a million cells per milliliter, in such circumstance they can produce harmful dinotoxins (Shimizu, 1978; Cembella, 1989). Dinoflagellate cyst assemblages are commonly used as paleo environmental indicators for reconstruction of sea-surface temperature, salinity, primary productivity, sequence stratigraphy, coastal eutrophication and pollution (Biffi and Grignani,1983; Haq et. al., 1987; Dale, 1996; de Vernal et al., 1997, Zonneveld et. al., 2001; Pospelova et. al., 2008; Durugbo et. al., 2013). Dinophyta and dinocyst studies of estuarine or brackish waters are rare or limited in

the Niger-Delta and most studies have focused on pelagic plankton or microalgae (Ogamba et. al., 2005 ; Edogbotu and Aleleye-Wokoma, 2009).

Chicoco mud is the local name of a dark-brown to pitch-black organic marine mud that superficially covers about 40% of the intertidal or littoral flat of the mangrove swamp in the Niger Delta region of Southern Nigeria (Omotosho, 2008). *Chicoco* mud can best be described as a soft grey organic fibrous clay which resembles wood and is usually located at or near the earth's surface. It is scientifically known as peat, however, the choice of *Chicoco*-mud rather than Peat was drawn from the fact that there is no universally accepted terminology to describe this environment, which reflects traditional differences in understanding and comprehension among specialist (IPS, 1984; Häkan and Jeglum, 2006).

In Nigeria's Niger Delta, there is a limited to no knowledge about dinophyta and dinocyst assemblage in *Chicoco* mud thus**,** knowledge of their assemblage composition and diversity becomes relevant in trying to understand its ecological significance within the littoral fringes of the New-Calabar River.

2. MATERIALS AND METHODS

2.1. The Study Area

This study area is located within three communities of the Asari-Toru Local Government Area (ASALGA) in the Kalabari Kingdom, Rivers State. Figure 1 shows the map of the study area which cuts across Buguma (BGM) through Ido (ID) to Abalama (ABL) communities and consisted of five Stations *viz* BGM 1, ID 2, ID 3, ABL 4 and ABL 5. ASALGA lies within the transition zone of the Niger Delta region and is bounded by Latitude 4^{o}40'N to 4^{o}50'N of the Equator and Longitude 6^{o}50'E to 6^{0}48'E of the Greenwich Meridian. The New-Calabar River is the major tidal river that flows through this Local Government Area, it is a tidal river with a highly productive mangrove community.

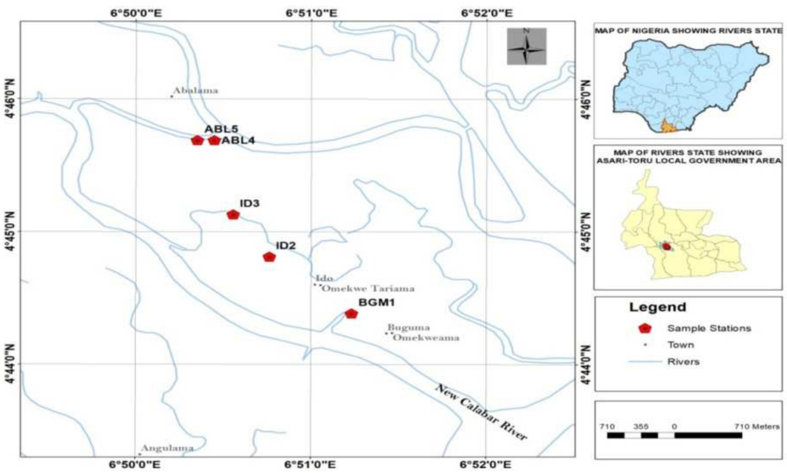


Figure 1: Map of Study Area Showing Sample Stations

2.2. Chicoco Mud Sampling and Analysis

The *Chicoco* mud was sampled with the aid of a 0.0625 m^2 Quadrat which was used to mark the mud surface and a Border spade with a solid forged head size of 0.0322 m^2 was used to excavate cubes measuring 0.014 m^3 at two different random points of a particular Station during Extreme Low Tide (ELT), all samples had two replicates. These cubes were put in labeled black cellophane bags before proceeding to the laboratory for *Chicoco* mud physico chemistry and benthic dinophyta and dinocyst analysis. One kilogram of the sampled cube was weighed, put in a plastic bowl and five liters of tap

water was added, this mixture was gently washed thoroughly, swirled, and decanted several times through a 0.05mm sieve so as to remove the plant fibers, while the sediment in solution was allowed to settle before decanting the clear water and collecting the sediment. This sediment sample was put in a polyethylene bag and air dried.

2.3. Cleaning and Concentration of Dinophyta and Dinocyst from Chicoco-Mud

A hundred grams of the sampled cube (surface to 10 cm depth) was weighed and put in a bowl, 100 ml of water was added to it to form a slurry and specific gravity was measured in order to ensure a consistent composition, this mixture was made up to 500mls and initially poured into two sieves (1mm and 0.5mm mesh sizes) so as to remove the fibers before finally pouring into six sieves of different sizes (200 µm, 177, 200, 63, 38, 20 µm) placed vertically in a serial order, refined with tap water controlled by a power hose to a clear consistency, the clean sediment retained in the 20 µm sieve size was removed from the rack and transferred into a 500ml beaker (Matsuoka and Fukuyo, 2000, 2003), 100ml of water was added, swirled and decanted into a 20ml vial container and preserved with neutral formalin (3% against the volume of the sample). This is the clean and concentrated or refined sample.

2.4. Identification of Dinophyta and Dinocyst Species

To this final refined sample, 0.02 ml of brilliant cresol green was added and one drop (0.01ml) was sub-sampled onto a glass slide, covered with a coverslip and examined with a binocular microscope (APHA, 1998) atx100 and x400 magnification, further examination at high power objective was also done to adequately identify the organism based on a planktological taxonomic system, either to species or genera (Rochon et. al., 1999 ; Matsuoka & Fukuyo, 2000). Identification to genera was effected only if a combination of several identification characteristics were not clearly visible (Matsuoka & Fukuyo, 2000).

2.5. Chicoco-mud Textural Analysis

One kilogram of the mud was weighed and put inside a plastic bowl and five liters of tap water was added. The mud was thoroughly washed and decanted using a 0.05 µm sieve so as to separate the fibers from the sediments. The wet fibers were weighed while the textural analysis of the sediment was carried out by adopting the method of Bouyoucos, 1951.

2.6. Sediment Physicochemical Analysis

The following analysis was carried out: Sediment pH, sediment textural class, total organic carbon, available phosphorus, nitrates, and sediment conductivity. Sediment pH was determined following the method described by Bates (1954), total organic carbon followed the method of Walkley and Black, 1934, available phosphorus was determined by the method of Bray and Kurtz (1945), nitrate followed the Brucine-Sulphate method (APHA, 1998 – Section 419 D) and wet sediment conductivity was carried out using a *pH* meter.

2.7. Biometry

Analysis of variance (ANOVA) was carried out on the data and the means of the data were presented along with ± SD (Standard Deviation) and separated using the Duncan Multiple Range Test (DMRT). Diversity indices adopted for this study were Margalef's Species Richness Index (d), Shannon-Wiener Diversity Index (H) and Evenness Index (E), while Hutcheson (1970) was used in calculating *T* so as *to* test for significant differences between sample H. The Coefficient of Variability (CV) was determined for the respective variables (Ogbeibu, 2005).

3. RESULTS

3.1. Physicochemical Parameters

Table 1 and 2 shows the physicochemical data of the study.

Table 1: Mean Monthly Variation (± SD & DMRT) of Physico-Chemical Data for Chicoco Mud

S/No	Variables	June	July	August	September	October	CV%
1.	Sand (%)	20.80±11.19a	11.00±5.96d	13.40±13.49c	15.00±8.86b	9.40±4.17e	31.0
2.	Silt (%)	5.60±3.38e	6.60±4.93d	22.60±35.90a	8.20±6.85b	9.00±4.29c	133.6
3.	Clay (%)	73.60±13.17d	82.40±10.67b	64.00±34.19e	76.80±12.27c	82.60±6.50a	20.2
4.	Fiber (g/kg)	215.86±44.81c	178.06±27.36e	189.52±53.82d	224.56±22.83b	240.48±30.46a	24.4
5.	Conductivity(µS/cm)	1186.60±144.0e	1449.40±193.2d	1517.80±447.3c	1842.60±405.2a	1635.00±347.9b	31.6
6.	TOC (mg/kg)	4.10±0.64e	5.13±0.85c	4.91±0.60d	5.24±0.35b	5.58±0.89a	22.2
7.	pH	4.57±0.32e	4.90±0.41c	4.92±0.90a	4.88±0.33d	4.92±0.39b	5.73
8.	Avail. P (mg/kg)	5.83±2.70e	13.18±5.06b	10.26±2.76d	13.07±5.69c	14.72±2.39a	61.4
9.	NO_3^- - N (mg/kg)	9.81±4.20e	11.73±2.87d	14.07±4.35b	15.14±7.24a	11.93±1.59c	33.4

Table 2: Mean Stations Variation (± SD & DMRT) of Physico-Chemical Data for Chicoco Mud

S/No	Variables	BGM 1	ID 2	ID 3	ABL 4	ABL 5	CV%
1.	Sand (%)	10.00±6.21d	14.60±14.14b	21.00±7.83e	21.00±9.20a	14.00±7.23c	29.7
2.	Silt (%)	8.20±6.26c	6.40±2.13d	22.40±6.10a	9.60±6.20b	4.40±2.67e	139
3.	Clay (%)	81.80±11.88a	79.00±14.68c	34.42±36.10e	69.40±9.39d	81.60±8.37b	58.1
4.	Fiber (g/kg)	220.98±54.26b	224.72±40.04a	218.80±25.15c	166.56±39.88e	217.42±24.69d	23.1
5.	Conductivity(µS/cm)	1740.20±285.09a	1521.20±351.05c	1508.00±425.60d	1334.80±114.96e	1527.20±512.97b	18.8
6.	TOC(mg/kg)	4.52±0.45e	5.02±0.82c	4.88±0.68d	5.22±1.13b	5.33±0.83a	12.7
7.	pH	4.83±0.49c	4.80±0.26e	4.81±0.35d	4.84±0.23b	4.92±0.40a	1.96
8.	Avail. P (mg/kg)	12.86±6.93b	12.33±5.82c	9.20±3.53e	13.43±3.91a	9.24±2.00d	35.7
9.	NO^-_3 – N (mg/kg)	12.64±7.09b	12.43±5.26c	11.80±4.28d	14.02±3.40a	11.79±2.67e	14.5

3.1.1. Wet Conductivity

The mean monthly and station conductivity variations indicated that monthly conductivity levels increased consistently during the study period, with values ranging between 1186.60±144.02 in June and 1842.60 ± 405.16 µS/cm in September and decreased in October while Stations conductivity variations ranged from 1334.40±114.96 in Station ABL4 to 1740.20 ± 285.09 in Station BGM1. The CV for monthly and stations conductivity data was 31.6 and 18.6 %.

3.1.2. Sediment Textural Analysis

The mean monthly and station variations showed that Sand ranged from 9.40±4.17 in October to 20.80±11.19 percent in June representing the highest value while for Stations it was highest in Station ABL 4 at 21.00± 9.20 and lowest in Station BGM1 [10.00 ± 621] and ID3 [10.00 ± 7.38]. The highest value of Silt was recorded in August (22.60±35.90) and the lowest value was in June (5.60±3.38), however, Station value recorded for Silt was 22.40 ± 36.10 in Station ID3 representing the highest value and the lowest value was recorded in Station ABL5. Clay content in the mud was highest in October (82.60±0.50) and lowest in August (64.00±34.19), while in Station ID3 it ranged from 67.60 ± 34.42 to 81.80 ± l11.88 percent in Station BGM1. Nevertheless, the fiber content decreased from 215.86±44.81 in June to 178.06±27.36 g/kg in July and gradually increased through the months and peaked in October (240.48±30.46) while variations in the fiber content was different with respect to the Stations and was highest in Station ID2 (224.72 ± 40.04] and lowest in Station ABL4 [166.56 ± 39.88]. Generally, the monthly Coefficient of Variability (CV) for Sand, Silt, Clay and Fiber was 31, 133.6, 20.2, and 24.4% while that for Stations was 29.7, 139, 58.1 and 23.1 % respectively.

3.1.3. Total Organic Carbon (TOC)

Mean monthly and stations variations of Total Organic Concentration (TOC) indicated that the TOC level was highest in October and lowest in June while Station ABL5 [5.33 ± 0.83) had the highest value and Station BGM1 the lowest[4.52 ± 0.45]. The CV for monthly and stations TOC data was 22.2 and 12.7 %.

3.1.4. pH

The pH values showed that Station 5 had the highest level [4.92 ± 0.40] and station 2the least level [4. 80±0.26]. The pH values recorded within the study sites showed that station 5 had the highest level [4.92 ± 0.40] and station 2 the least level [4. 80± 0.26]. The CV for monthly and stations pH data was 5.73 and 1.96 %.

3.1.5. Avail. P and Nitrate (NO_3 - N)

The variation in PO_4 concentration was higher in the month of October (14.72 ±2.39) and lower in June (5.83±2.70). The NO_3 values during the study period increased through the months and peaked in September L 1.4 ± 7.24). Thereafter the level reduced to 11.93 ± 159 in October. The lowest value was recorded in June (9.81±4.20). The phosphates concentration showed a decreasing trend between station 1 [12.86 ± 6.93] and station 3 [9.20 ± 3.53]. The level increased to 13.43 ± 3.91 in station 4 representing the highest level within the study sites. The nitrate concentration ranged from 11.79 ± 2.67 in station 5 to 12.64 ± 7.09 in station 1. The CV for monthly Avail. P and Nitrate (NO_3 - N) data was 61.4, and 35.7 % while for stations it was 33.4 and 14.5 %.

3.1.6. Dinophyta and Dinocyst Diversity and Abundance

Figure 2 (A, B, & C) and 3 (D & E) shows the dinophyta and dinocyst variation during the study period while the ecological indices (*d*, *H*, *E*) are indicated in inset form on the graphs. The abundances of dinophyta and dinocyst varied differently within and between stations. Assemblage composition amongst the dinophyta was the 'resting cyst' producing species of *Polykrikos schwartzii* Bütschli (Gymnodiniales) and *Preperidinium meunieri* (Pavillard) Elbrächter (Peridiniales) while for the dinocyst it was *Protoperidinium* spp. (Peridiniales), which was not identified to species level due to the orientation and folding of the cyst walls. In this study the dinocyst was higher in number than the dinophyta in most of the sampling stations resulting in varying collective abundances of 57 and 43% in June and 69 and 31% in October, however, this pattern was different in August with the dinocyst having 48% and dinophyta,52%.

The ecological indices indicate that mean Margalef's species richness index (*d)* varied from 0.243 in July to 0.422 in June while Shannon-Weiner diversity index (*H*) varied from 0.249 to 0.400 bits/individual in September and October, furthermore, the evenness or equitability index (*E*) varied from 0.537 to 0.931 in July and October. However, Hutcheson *T*-test for comparing significant differences between station's *H* indicated significant and not significant differences at $p < 0.05$ level, for instance, *H* of stations BGM 1vs ID 2, ID 2 vs ABL 5 were not significant while *H* of ID 2 vs ABL 4, ABL 4 vs ABL 5, BGM 1 vs ABL 5 and BGM 1 vs ABL 4 were all significant.

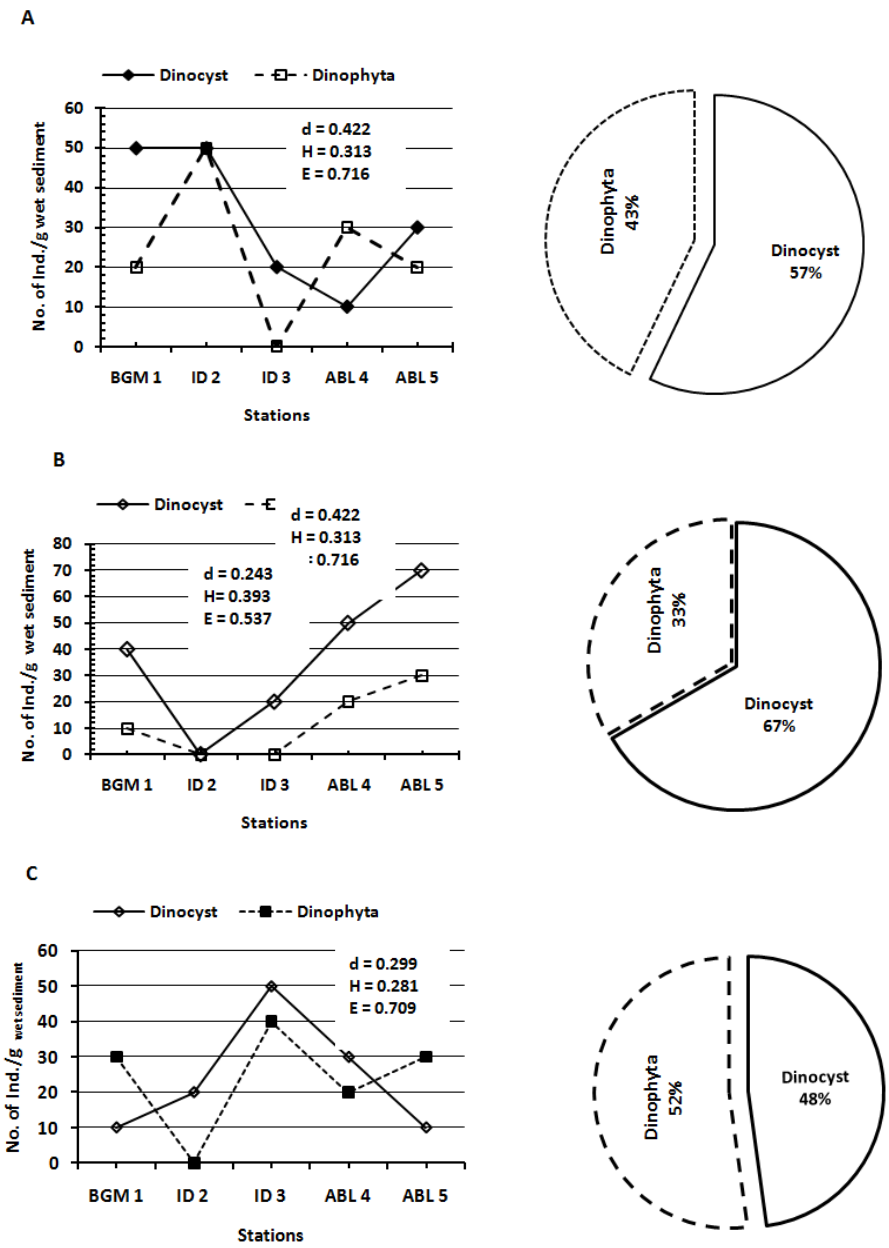
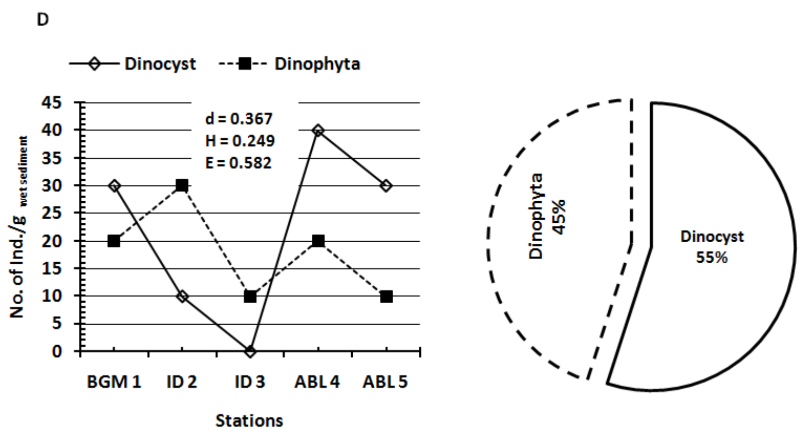


Figure 2: The Dinophta and Dinocyst Variation: A. June, B. July and C. August; Inset is the Mean Margalef's Species Richness (d), Shannon-Wiener Diversity (H) And Evenness Index (E) For Stations



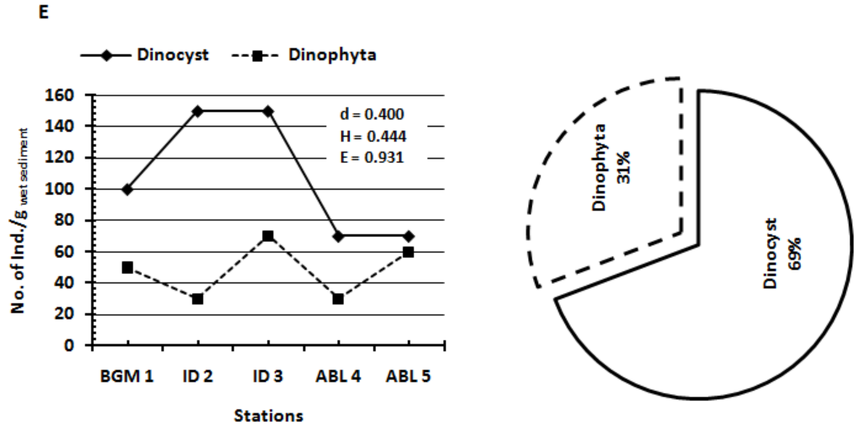


Figure 3: The Dinophyta and Dinocyst Variation: D. September and E. October; Inset is the Mean Margalef's Species Richness (d), Shannon-Wiener Diversity (H) And Evenness Index (E) For Stations

4. DISCUSSIONS

The diversity and abundance of littoral dinophyta and dinocyst varied within the period of the study, assemblage composition amongst the dinophyta were the 'resting cyst' producing species of *Polykrikos schwartzii* Bütschli (Gymnodiniales) and *Preperidinium meunieri* (Pavillard) Elbrächter (Peridiniales) while for the dinocyst it was *Protoperidinium* spp. (Peridiniales), which was not identified to species level due to the orientation and folding of the cyst walls. One of the challenges of this study was the dearth of information on benthic dinophyta and dinocyst studies in the Niger Delta, however, studies (McMinn, 1989; Hallegraef and Bolch,1992) have been carried out in tropical, subtropical and temperate environments. Dinocyst assemblages show variability within estuaries caused by differences in nutrient supply, water mixing, salinity, and sea surface temperature, even at small spatial scales (Pospelova et al., 2004). In this study, the dinocyst was higher in number than the dinophyta by 55 to 69% despite their low diversity and abundance, which was inconsistent with previous studies (Matsuoka et. al., 1998, Amorim & Dale 1998).

Stations' variations indicated that the average Margalef's Species Richness Index ('d') was very low, while Evenness or Equitability Index ('E') which is usually constrained between 0 and 1.0 showed species were not evenly abundant in all stations, however, the Shannon-Weiner Diversity Index ('H') indicated that diversity of dinophyta and dinocyst were minimal and did not show any appreciable seasonal variation.

The significant differences in species diversity index as provided by Hutchinson (1970) indicated that diversities between stations varied, for instance, there was no significance difference between the diversities of some stations while in other stations significant differences occurred. These variations may have been caused by the effects of the physicochemical variables, for instance, the sediment texture of *Chicoco*-mud was made up of sand, silt, clay and plant fiber which significantly affected their diversity and abundance as evident from the DMRT values, however, the stability of these physicochemical variables were different considering their Coefficient of Variability (CV) values, which confirmed that fiber was a less variable parameter and hence more stable in the intertidal environment than Clay, Sand and Silt and had specific significant effects, similarly, monthly variations indicated that fiber and clay components were the most stable unlike Sand and Silt. Thus, the presence of fiber and clay may play a significant role in the diversity and abundance of littoral dinophyta and dinocyst which corroborates the observation of Anderson *et. al.,* 1995, however, the role of fiber was not clearly understood but may function as a source of the nutrient.

Monthly and station values for Conductivity, Total Organic Carbon (TOC), pH, Available Phosphorus (Avail. P.,) and Nitrate-Nitrogen (NO^-_3 – N) indicate that pH was the least variable parameter and the most stable followed by TOC, NO^-_3 – N, and Avail. P., high CV values for NO^-_3 – N, and Avail. P was consistent with their characteristic nature (absorption and release) in the environment of been regularly absorbed and released by organisms in the environment. Similarly, the mean stations' 'DMRT' values showed that their effects were significant on the diversity and abundance of littoral dinophyta and dinocyst except in some stations. Some of these physicochemical variables affect the diversity and abundance of littoral dinophyta and dinocyst either by acting as a limiting or regulatory factor, however, Eltringham (1971) posited the importance of distinguishing between limiting and regulatory factors, thus, the pH and TOC concentration which was less variable parameters and generally stable in the environment could be considered as a regulatory factor because they controlled the size of the population of dinophyta and dinocyst without destruction while NO^-_3 – N, and Avail. P. were limiting factors because they may slow or prevent the population from spreading, nevertheless, a regulatory factor can under certain circumstances be a limiting factor and vice versa.

The acidic nature of the *Chicoco* mud observed in this study was consistent with other studies (Wheatley, et. al., 1996) but the levels of electrical conductivity were different due to varying localized conditions. Nevertheless, Joosten and Clarke (2002) reported that variations in pH, could be linked also to electrical conductivity, calcium content, and base richness, which may affect the availability of plant nutrients, although, the effects of calcium content and base richness were not investigated, the other two parameters were capable of reducing nutrients thereby reducing their population. However, for microorganisms, a key factor was the nature of the 'Chicoco mud' (peat), specifically its botanical composition and its quality (i.e. the amount of easily decomposed organic matter). In fact, the peat type and amount of quality organic matter were undoubtedly the most important factors influencing rates of decomposition, biomass growth, and diversity (Wheatley et. al., 1996). This may also have contributed to the low diversity and abundance of littoral dinophyta and dinocyts in this study.

5. CONCLUSIONS

This study has proved the presence of littoral dinophyta and dinocyst in *Chicoco* mud, which was composed of two taxa namely Gymnodiales and Peridinales with the dinocyst been more abundant but less diverse than the dinophyta, thus, *Chicoco* mud is a sensitive habitat that should be conserved for future ecological studies and monitoring so as to better understand this environment for sustainable management. The species diversity and abundance were very low and was either significant or not significant which may have been caused by variations in physicochemical parameters and the clay-fiber complex.

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